

Morphologic correlates of the multimodal electromagnetic exposures efficiency (as exemplified by the experiment tumor growth)

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Aims

The aim of the present paper is to study the morphofunctional changes in the tumor tissue and peripheral zone in experimental animals under the influence of extremely high-frequency electromagnetic radiation (EHF EMR). Combined effect of EHF EMR and ultra-low-frequency magnetic field (ULF MF) without the use of special anti-tumor agents is to be studied as well.

Materials and methods

The experiments have been performed on 56 outbred male rats with inoculated sarcoma 45. Low intensity EHF EMR and ULF MF exposures have been carried out only on animals' heads. Histological and morphometric study of the sarcoma 45 tumor tissue and its peripheral area has been performed.

Results

In case of the combined electromagnetic exposure (in 65 % of cases) against the background of the stable anti-stressor adaptation reactions an evident anti-tumor effect has been observed. Under effective combined electromagnetic action, lymphocyte infiltration of the tumor tissue and its peripheral zone was enhanced not only in comparison with the reference group, but also compared to the EHF EMR monoexposure.

Conclusions

Thus, significant enhancement of the tumor tissue infiltration by leukocyte cells under the combined effect of EHF EMR and ULF MF may indicate mobilization of the local immune mechanisms due to the activation of the neuroendocrine and immune system organs.

Keywords

Electromagnetic exposure, Anti-tumor effect, Tumor infiltration,
48 | *Cardiometry* | Issue 11. November 2017

Anti-stressor adaptation reaction

Imprint

Tatiana A. Barteneva, Alla I. Shikhlyarova, Galina Zhukova, Tatiana P. Protasova, Marina I. Bragina, Elena A. Shirnina. Morphologic correlates of the multimodal electromagnetic exposures efficiency (as exemplified by the experiment tumor growth). *Cardiometry*; Issue 11; November 2017; p.48–53; DOI: 10.12710/cardiometry.2017.11.4853; Available from: <http://www.cardiometry.net/issues/no11-november-2017/multimodal-electromagnetic-exposures-efficiency>

Introduction

Recently, the methods of low-intensity electromagnetic therapy are increasingly used in clinical oncology as a component of complex anti-tumor treatment [1–13]. It has been shown that the effects of weak electromagnetic radiations, both ultra-low and extremely high-frequency range, on the CNS structures can provide a pronounced anti-tumor effect [1–13]. At the same time, one of the mechanisms of the therapeutic effect of weak electromagnetic radiation is the development of stable anti-stressor adaptation reactions (AR) with calm and increased activation [14, 15], which intensify the activity of the neuroendocrine and immune system organs. At present, the effector mechanisms of mediated damaging action of weak electromagnetic exposures on the tumor tissue remain poorly studied. In this connection the aim of the paper is to study the morphofunctional changes in tumor tissue under effective electromagnetic exposures used in the modes of activation therapy.

Materials and methods

The experiments have been conducted in 56 albino outbred male rats, weighing 200–250 g and bred in the vivarium of the Rostov Research Institute of Oncology. As a tumor model, an inoculated sarcoma 45 (C-45) strain, obtained at the N.N. Blokhin Russian Cancer Research Center, RAMS, Moscow, has been used. The studies have been carried out in accordance with the International Rules and bioethical norms for working with laboratory animals [16]. The tumors have been inoculated by subcutaneous injection into the region of the back inferolateral surface of 0,3 ml of a tumor cells suspension. When the tumor reached the volume of 0.7–1.2 cm³, the exposures have been

initiated using the Yav-1 device (42.2 GHz, 10 mW/cm²), the induction coil and the purpose designed modulator based on a special-shaped signal generator. Under the combined action the modulated millimeter radiation (EHF EMR) and the ultra-low frequency magnetic field (ULF MF) radiation of small induction (1 mT) has been simultaneously used. The frequency of the ULF MF varied in the range of 0.8–7.8 Hz in accordance with the EHF EMR modulation mode. The animal's head has been exposed to the actions [1, 2]. The sessions have been conducted 4–5 times a week during 4 weeks.

The tumor volume has been determined 2 times a week. The tumor linear dimensions have been measured in three mutually perpendicular planes. Then its volume has been determined by the formula $V = (a \cdot b \cdot c) \cdot \pi / 6$, where a, b, c are the linear dimensions of the tumor in cm, V is the tumor volume.

At the end of the experiments, the animals were sacrificed by means of an etheric over-narcotization. The 10% solution of neutral formalin and the Carnoy's fixative have been used to fix the material. The tumor sections have been stained with hematoxylin-eosin and according to the Brache method. Morphometry of the tumor tissue and its peripheral zone has been conducted with the help of the automated measuring system "SAGA" on the basis of the PC. The study of the cellular composition of the tumor has been performed with an increase of x by 1000. The number of lymphocytes and plasmocytes in each 10th field of vision per 1000 cells has been determined in the tumor tissue (incr. 1000). In the peripheral zone of the tumor counted has been the number of macrophages, lymphocytes, plasmocytes, tissue basophils in each 5th field of vision, and then the average number has been determined.

As a signal indicator of the AR type and intensity, the blood leukocytes formula counted for 200 cells has been used [14, 17]. The AR condition has been determined before the beginning of the course of actions, during the experiment (1 or 2 times a week) and on the day of the rats slaughter.

The statistical analysis of the study results has been carried out with the help of t-criterion by Student and the Wilcoxon-Mann-Whitney criterion.

Result and discussions

During the experiment an active growth of C-45 has been observed in the reference group of rats. Under the action of the polyfrequently modulated EHF EMR

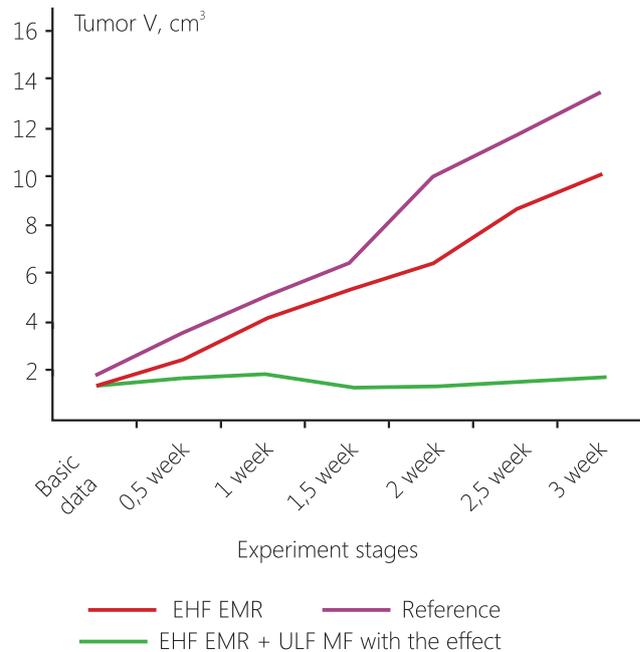
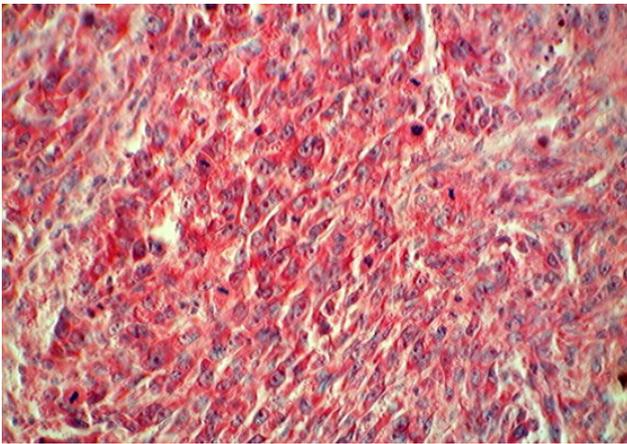


Fig.1. Dynamics of the sarcoma 45 size under different modes of electromagnetic therapy in albino outbred male rats: Reference group; group with the EHF EMR exposures; EHF EMR + ULF MF – subgroup of animals with the evident anti-tumor effect; EHF EMR +ULF MF – subgroup of animals without the effect.

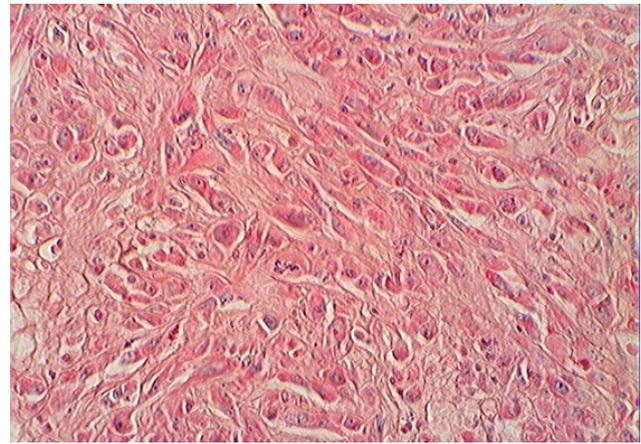
as a monofactor, a slow (by 36 %, $p < 0.05$) inhibition of sarcoma 45 growth has been observed 2 weeks after the beginning of the exposures. This inhibition turned out to be Table and a week later the differences ceased to be reliable in comparison with the tumor size in rats of the reference group (Fig. 1).

In the group of animals receiving the combined electromagnetic effect ($n = 20$), a significant anti-tumor effect has been observed in a part of the rats. It has allowed distinguishing in the group two subgroups of animals that had significant differences in the dynamics of tumor size. In the first subgroup (EHF EMR + ULF MF1, Figure 1), consisting of 13 animals (65 % of animals in the group), a pronounced inhibition of sarcoma 45 growth and even tumor regression in 4 cases have been observed. In the 2nd subgroup (EHF EMR + ULF MF2, Fig. 1), consisting of 7 rats (35 % of animals in the group), no anti-tumor effect has been observed.

The histological examination of the tumor tissue in the reference group animals has showed marked signs of its intensive growth with violation of the capsule integrity and sprouting into the neighboring tissues. Only small groups of dystrophically altered tumor cells have been noted. The infiltration of the tumor tissue by the immune system cells has also been poorly pronounced (Fig. 2, Table 1).



A)



B)

Fig. 2. Sarcoma 45. A. Reference group. Intensive growth of the tumor, dense arrangement of cells, numerous figures of mitosis. B. EHF-therapy. Extensive areas of dystrophically altered tumor cells (pycnosis of nuclei, vacuolation of cytoplasm), decrease in the density of their arrangement. Brache. Magn. x 400

Micropicture of sarcoma 45 under the EHF EMR action has indicated the development of degenerative-dystrophic changes in some cells and a tendency to decrease in their density that led to only an unstable inhibition of the tumor.

In cases of the most pronounced anti-tumor effect of the combined action, i.e. regression of sarcoma 45, significant growth of connective tissue infiltrated by immune competent cells has been observed at the site of the regressed tumor.

The data on the immune system cells, located in sarcoma 45 tissue in the studied group rats (Table 1), were of great interest. Both effects contributed to intensification in lymphocyte infiltration of the tumor tissue. The number of tissue-infiltrating immune cells has increased in both the EHF EMR and the combined electromagnetic effect groups.

Thus, in animals of the EHF EMR monoaction group the number of lymphocytes increased by 2,3 times, and the number of plasmocytes increased by 4,6 times as compared with the reference group (Table 1). Under the combined electromagnetic exposure in the 1st subgroup in cases of the tumor growth inhibition the number of lymphocytes in tumor has been 3,8 times higher compared with the reference, and 1,6 times higher than in the group where only the EHF EMR has been applied (Table 1). The plasmatic cell number has increased 4,7 times on the average under the EHF EMR monoaction. Under the combined exposure it has increased 6,7 times in the 1st subgroup in case of inhibition of the tumor growth and by 4,0 times in the 2nd subgroup of animals as compared with the reference (Table 1).

In the peripheral tumor area the largest number of lymphocytes, plasmocytes and tissue basophils has been observed under the combined electromagnetic exposure (EHF EMR + ULF MF) in the 1st subgroup of animals. In the reference group rats a minimal content of the immune system cells has been observed in the tumor periphery: single lymphocytes and low frequency tissue basophils; no plasmocytes and macrophages observed.

In the EHF EMR group the number of lymphocytes in the peripheral tumor area increased by 7,8 times as compared with the reference group. In the 2nd subgroup of animals receiving the combined exposure, the content of lymphocytes (and the tumor micropicture as a whole) has not differed significantly from the EHF EMR monoaction group (Table 2).

Under the combined exposure in the 1st subgroup, where the lymphocytes number has been maximal (Fig. 2), the given marker has 8,3 times exceeded the reference values and differed (reliably or on a tendency level) from the markers in the 2nd subgroup and the EHF EMR monoaction group (Table 2). In cases of the sarcoma 45 growth inhibition under the effect of EHF EMR and ULF MF in peripheral part of the tumor a tendency to an increase in the lymphocytes mean number in the field of vision has been observed.

Thus, the higher is the exposure efficiency the more significant is the content of lymphocytes in the tumor periphery (Fig. 3).

Besides, under the studied electromagnetic exposures observed has been appearance of macrophages and plasmocytes in the peripheral tumor area (Fig. 3), that has not been noted in the reference group.

Table 1. Markers of sarcoma 45 tissue infiltration in albino outbred rats by the immune system cells under the EHF EMR action and the evident tumor growth inhibition under the influence of combined electromagnetic exposure (EHF EMR + ULF MF)

Marker		Reference (n=16)	EHF EMR (n=20)	EHF EMR + ULF MF	
				s/gr. 1, tumor growth inhibition (n=9)	s/gr. 2 (n=7)
Weight of tumor, g		16,5±1,5	13,6±1,0	2,0±+0,3 ●□▼	14,5±1,1
Tissue of tumor	Number of lymphocytes (‰)	34,54±2,63	80,82±10,47 ●	130,86±13,15 ●□	110,63±18,5 ●
	of tumor	0,74±0,42	3,45±1,08 ●	4,95±1,02 ●	2,98±1,03 ●

Note: Differences in relation to: reference ● – $p < 0,05-0,001$; from the EHF EMR group: □ – $p < 0,05$; from subgroup 2 EHF EMR + ULF MF: ▼ – $p < 0,05$, Field of the vision area – 6787,12 μm^2 . EMR EHF + ULF MF is a subgroup of animals with an evident anti-tumor effect

Table 2. The content of immune cells in sarcoma 45 peripheral area in albino outbred rats under the EHF EMR action and the evident tumor growth inhibition under the influence of combined electromagnetic exposure (EHF EMR + ULF MF)

Number of immune cells in the field of vision	Reference (n=16)	EHF EMR (n=20)	EHF EMR + ULF MF	
			s/gr.1, tumor growth inhibition (n=9)	s/gr. 2 (n=7)
Weight of tumor, g	16,5±1,5	13,6±1,0	2,0±+0,3 ●□▼	14,5±1,1
Macrophages	0	4,5±0,9 ●	5,9±0,9 ●	5,0±1,0 ●
Lymphocytes	3,2±0,3	23,5±1,1 ●	26,9±1,3 ●T□▼	21,9±1,2 ●
Plasmocytes	0	4,6±0,6 ●	6,5±0,2 ●□▼	5,1±0,3 ●
Tissue basophils	0,16±0,06	0,21±0,04	0,49±0,06 ●□▼	0,22±0,04

Note: Differences in relation to: reference ● – $p < 0,05-0,001$; from the EHF EMR group: □ – $p < 0,05$, T□ – $p < 0,1$; from s/gr 2 EHF EMR + ULF MF: ▼ – $p < 0,05$. Field of vision area – 6787.12 μm^2 . EHF EMR+ULF MF – combined action of poly-frequency modulated EHF EMR and ultra low frequency magnetic field (ULF MF)

The plasmocytes content in the rats of the 1st subgroup with the tumor growth inhibition under the effect of the combined exposure has exceeded the number of these cells in the field of vision by 27 % and 41 % in the animals of subgroup 2 and the EHF EMR monoaction group, respectively (Table 2). The macrophage content in the tumor peripheral part has not reliably differed in the observed groups. At the same time it should be noted that this marker has reached its maximum value (up to 10 cells in the field of vision) in rats with tumor regression under combined action of EHF EMR and ULF MF.

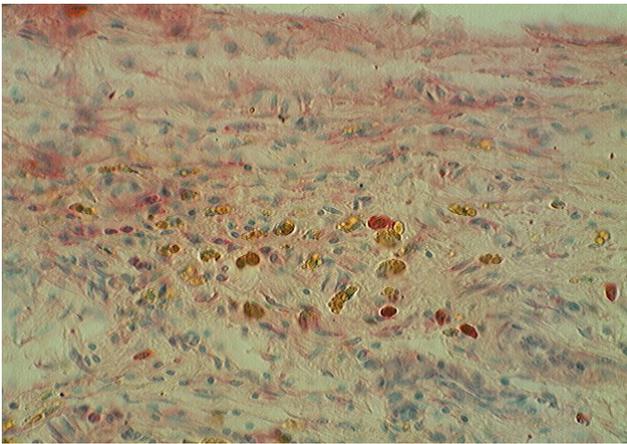
The tissue basophil content in the tumor peripheral area has reliably increased as compared with this marker value in the reference group only in cases of effective combined electromagnetic exposure (subgroup 1). The mean number of tissue basophiles (in the field of vision) in animals with tumor growth inhibition under the influence of combined exposure has been almost 3 times

higher than in the reference group rats, and more than 2 times exceeded this marker in the 2nd subgroup rats and the animals exposed to only EHF EMR (Table 2).

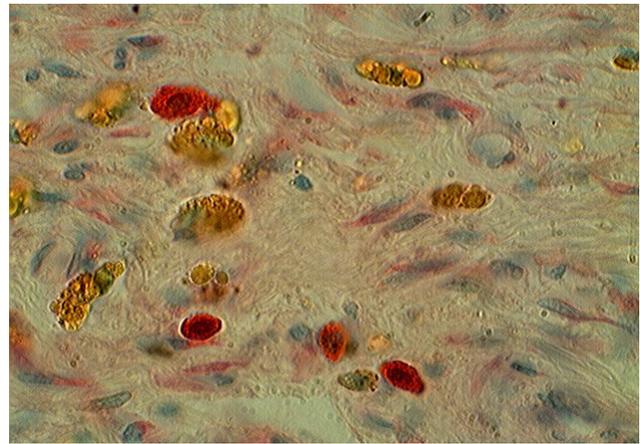
Conclusions

Thus, the obtained results indicate high anti-tumor efficiency of electromagnetic exposures under their use in the activation therapy modes. Low intensity and localization of exposures (to animal's head) precluded the possibility of their direct damaging effect on tumor cells. At the same time significant enhancement in the tumor tissue infiltration by leukocyte cells may denote mobilization of the local immune mechanisms due to activation of neuroendocrine and immune system organs.

The tumor tissue histological and morphometric analysis under the studied exposures indicate their mediated damaging effect on sarcoma 45 cells. Moreover, the most significant effect has been noted under combined use of



A)



B)

Fig. 3. Sarcoma 45. Combined action of EHF EMR and ULF MF. Partial regression. Infiltration by immune cells – lymphocytes, plasmocytes and macrophages. Brache. A. Magn. x 400. B. Magn. x 1000

EHF EMR and ultra low frequency magnetic field. At the same time the qualitative and quantitative composition of the immune system cells infiltrating the tumor tissue has corresponded to the anti-tumor effect intensity. The appearance under effective exposures of plasmocytes and macrophages, which have been practically absent in the reference group animal tumors, as well as a reliable increase in tissue basophil content, may indicate immediate participation of the noted immune system cells in the mechanisms leading to tumor cells damage.

Thus, combined electromagnetic exposure (in case of appearance of s. Table increased activation AR), as opposed to the EHF EMR monoaction, has provided effective regulatory influences of the neuroendocrine system structures on the immune system ones and led to evident tumor tissue damage, mediated by the immune system effector elements.

Statement on ethical issues

Research involving people and/or animals is in full compliance with current national and international ethical standards.

Conflict of interest

None declared.

Author contributions

The authors read the ICMJE criteria for authorship and approved the final manuscript.

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